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Cell type- and region-specific enhancement of adult hippocampal neurogenesis by daidzein in middle-aged female mice

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ABSTRACT

Adult hippocampal neurogenesis is associated with various brain functions, such as learning, memory, and emotion. Intriguingly, reduction in new cell production in the hippocampus in middle age may underlie some of the cognitive deficits. Among several factors that may affect adult hippocampal neurogenesis, estrogens have been suggested to be critically involved in the cognitive impairment of postmenopausal women. Phytoestrogens, such as daidzein and genistein, are expected to work as estrogen substitutes. In this study, we aimed to clarify the effects of daidzein on adult hippocampal neurogenesis using middle-aged (12-month-old) female mice. Animals received daily intraperitoneal injections of daidzein or vehicle for four weeks, and the cells at specific stages of neurogenesis were presumptively defined using molecular markers. Administration of daidzein did not affect the numerical densities (NDs) of primary progenitors, early transient amplifying progenitors (TAPs), and astrocytes. In contrast, the NDs of late TAPs, neural progenitors, and immature granule cells were increased by daidzein. The NDs of proliferating cells, but not apoptotic cells, were also increased by daidzein. To examine the effects of daidzein on maturation of adult-born cells, we three-dimensionally traced their dendritic arbors: the branch number, total length, and intersection number (Sholl analysis) of immature granule cells were increased by daidzein. In general, the effects of daidzein were more dominant in the dorsal region than in the ventral region. The cell type- and region-specific enhancement of adult hippocampal neurogenesis by daidzein provides a key to understanding the actions of estrogen substitutes for the treatment of postmenopausal women.

1. Introduction

Increasing evidence indicates that the hippocampus is divided into multiple domains along its longitudinal and transverse axes (Chawla et al., 2005; Fanselow and Dong, 2010; Gallitano et al., 2016). Differentiation along the longitudinal axis of the hippocampus (dorsal region vs. ventral region) has been well studied, especially in rodents: the dorsal region plays an essential role in learning and memory, while the ventral region is involved in anxiety-related behaviors (Bannerman et al., 2003; Moser et al., 1995). In the dentate gyrus of rodents, differentiation along the transverse axis (suprapyramidal blade vs. infrapyramidal blade) has also been reported: granule cells are more active in the suprapyramidal than in the infrapyramidal blade during spatial task (Marrone et al., 2012; Ramirez-Amaya et al., 2005).

Throughout life, production of new granule cells continues in the dentate gyrus of the hippocampus (Altman and Das, 1967; Cameron et al., 1993; Kaplan and Hinds, 1977). It has been well accepted that adult hippocampal neurogenesis contributes to various brain functions (Balu and Lucki, 2009). For instance, proliferation of granule cells is essential for hippocampus-dependent spatial memory tasks (Dupret et al., 2007; Shors et al., 2001). It has also been suggested that new granule cells are required for mood control and anti-depressant efficiency (Petrik et al., 2012).

Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; bHLH, basic helix-loop-helix; BMP, Bone morphogenetic protein; BSA, bovine serum albumin; CB, calbindin; CR, calretinin; DAPI, 4′,6-diamidino-2-phenylindole; DCX, doublecortin; DMSO, dimethyl sulfoxide; ER, estrogen receptor; EE, enriched environment; FITC, fluorescein isothiocyanate; GFAP, glial fibrillary acidic protein; HSD, honestly significant difference; Mash1, mammalian achaete-scute homolog-1; MCM2, minichromosome maintenance 2; ND, numerical density; NeuN, neuronal nuclei; PFA, paraformaldehyde; PBS, phosphate buffered saline; PPARγ, peroxisome proliferator-activated receptor γ; Sox2, sex determining region Y-box 2; TAP, transient amplifying progenitors.

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Additionally, experience-induced alterations in the production of new granule cells may fine-tune the hippocampus to a predicted environment (Opendak and Gould, 2015).

The rate of new cell production in the hippocampus wanes radically during aging (Cameron and McKay, 1999; Kuhn et al., 1996; Seki and Arai, 1995). Age-related reduction in adult hippocampal neurogenesis is accompanied with the cell type- and region-specific changes (Jinno, 2011a; Walter et al., 2011). Aged animals with preserved spatial memory exhibit a higher levels of hippocampal neurogenesis, while those with spatial memory deficits showed a reduction in hippocampal neurogenesis (Drapeau et al., 2003). Retroviral gene delivery of the basic helix-loop-helix (bHLH) transcription factor (NeuroD1) into neural stem cells enhances adult hippocampal neurogenesis and rescue spatial memory in a mouse model of Alzheimer's disease (Richetin et al., 2015). Furthermore, alterations in adult hippocampal neurogenesis may also be involved in cognitive dysfunction clinically observed in aged people (Mesulam, 1999).

Estrogens play pivotal roles in the regulation of reproductive functions a via estrogen receptors (ERs) in the diverse systems of the body (Turgeon et al., 2004). Interestingly, a number of previous papers have reported positive effects of estrogens on cognitive functions (Gillies and McArthur, 2010; Hara et al., 2015; McEwen et al., 1997). In this regard, it should be noted that estrogens also affect adult hippocampal neurogenesis (Galea et al., 2013; Mahmoud et al., 2016). For example, estradiol replacement restores new cell productions in the dentate gyrus of ovariectomized rats, a surgical model of menopausal status (Tanapat et al., 2005). Continuous administration of estradiol benzoate increases cell proliferation and decreases cell death in the dentate gyrus of female but not male rats (Barker and Galea, 2008).

Phytoestrogens are plant derived compounds that exhibit biological functions comparable to estrogens (Patisaul and Jefferson, 2010; Whitten et al., 2002). Although phytoestrogens consist of a variety of compounds, they can largely be divided into three classes: isoflavones, lignans, and coumestans (Murkies et al., 1998). Isoflavones, e.g., daidzein and genistein, are mainly included in soy beans, lignans are present in flaxseed, and coumestans are found in clover and soybean sprouts (Sirotkin and Harrath, 2014). After ingestion, phytoestrogens are hydrolyzed by microbes in the intestine, absorbed and conjugated in the liver, circulated in the blood, and also excreted into the intestine via the bile (Cassidy, 2003; Day et al., 1998). In the gut, phytoestrogens are further metabolized by microflora into more active compounds, e.g., equol (Axelson et al., 1984) and *O*-Desmethylangolensin (Joannou et al., 1995).

Similar to estrogens, isoflavones are shown to improve cognitive function in rodents (Kohara et al., 2015; Neese et al., 2010; Zhao et al., 2015) and enhance the adult hippocampal neurogenesis (Rivera et al., 2013). In this study, we examined the effects daidzein on adult hippocampal neurogenesis in middle-aged female mice. Our observations provide a new insight into how phytoestrogens can be useful for postmenopausal women.

2. Materials and methods

2.1. Animals

In all, 16 middle-aged (12-month-old, 30–35 g body weight) female C57BL/6J mice (Kyudo KK, Tosu, Japan) were used in this study. Animals were housed on a 12/12-h light/dark cycle and fed with a standard rodent chow (CE-2; CLEA Japan, Tokyo, Japan) ad libitum. Every procedure was approved by the Committee of Ethics on Animal Experiments in the Graduate School of Medical Sciences, Kyushu University.

2.2. Administration of daidzein

A recent study has shown that intraperitoneal (i.p.) administration of daidzein (50 mg/kg, 13 consecutive days) promoted the adult hippocampal neurogenesis in middle-aged male Wistar rats (Rivera et al., 2013). To define the effects of longer-term administration of lower-dose daidzein on adult hippocampal neurogenesis in middle-aged female C57BL/6J mice, the following experimental conditions were used throughout the study: mice were given a daily i.p. administration (28 consecutive days) of daidzein (25 mg/kg; Tokyo Chemical Industry, Tokyo, Japan; $n = 8$) dissolved in 1% dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO) or vehicle (1% DMSO, $n = 8$) in 0.1 ml of saline. Because bioavailability and metabolism of isoflavone may differ between rats and mice, as well as between male and female (Sepehr et al., 2007; Soukup et al., 2016), the differences between vehicle controls and daidzein-treated mice are carefully evaluated in this study.

It might also be noted that the chow used for both vehicle controls and daidzein-treated mice is a soy-based diet (CE-2; CLEA Japan), which contains daidzein (157 μg/g) (Ashby et al., 2003; Brown and Setchell, 2001). Because the average daily consumption of feed for an adult mouse is 3–5 g, the estimated oral intake (per os, p.o.) of daidzein is 0.5–0.8 mg per day. On the other hand, the amount of i.p. administration of daidzein is 0.8–0.9 mg per day, because we used mice with 30–35 g body weight. Although the amount of daily daidzein intake is rather comparable between the two routes, it has been reported that the plasma levels of isoflavone (genistein) resulting from i.p. administration are 5 times higher on average than achieved by the p.o. route (Supko and Malspeis, 1995).

2.3. Tissue sectioning

Mice were killed with an overdose of sodium pentobarbital (120 mg/kg, i.p.), and perfused transcardially with phosphate-buffered saline (PBS, pH 7.4), followed by a mixture of 4% paraformaldehyde (PFA) and 0.05% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were left in situ for 2–3 h at room temperature and then removed from the skull. The brains were cut coronally into 40-μm-thick sections on a vibrating microtome (VT1000S; Leica Microsystems, Wetzlar, Germany). Coronal and horizontal sections were used to analyze the dorsal and ventral hippocampus, respectively (Fig. 1A). To avoid deformation of the sections, they were processed free-floating with extreme caution.

2.4. Molecular markers

To presumptively define the cells at specific stages of adult hippocampal neurogenesis, we used the following molecular markers: sex determining region Y-box 2 (Sox2) and glial fibrillary acidic protein (GFAP) to label neural stem cells (Brazel et al., 2005; Garcia et al., 2004), mammalian achaete-scute homolog-1 (Mash1) to label early transit amplifying progenitors (early TAPs) (Uda et al., 2007), minichromosome maintenance 2 (MCM2) to label early and late TAPs (Maslov et al., 2004), doublecortin (DCX) to label neural progenitors and immature granule cells (des Portes et al., 1998), calretinin (CR) to label postmitotic immature granule cells (Brandt et al., 2003), calbindin (CB) to label mature granule cell (Rami et al., 1987), Ki67 to label proliferating cells (Gerdes et al., 1984), caspase-3 (CASP3) to label apoptotic cells (Liu et al., 1997), S100β to label astrocytes (Liu et al., 1996; Raponi et al., 2007), spinophilin to label dendritic spines (Allen et al., 1997), and synaptophysin to label presynaptic terminals (Wiedenmann and Franke, 1985).